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DIASTASE ACTIVITY IN RELATION TO STAGE OF DEVELOPMENT AND CARBOHYDRATE CONTENT OF THE TUBER OF *SOLANUM TUBEROSUM*¹

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The effect upon enzyme activity of various factors, such as temperature, light, and different concentrations of salts, acids, and alkalis, has been studied in considerable detail, and the behavior of the enzymes, with respect to these factors, has thrown much light upon physiological processes. One phase of this question, however, has been touched upon only to a slight extent, that is, the relation of enzyme activity to the various stages of growth of plant organs. For this reason, it was thought that a study of the activity of the enzyme diastase, in relation to tuber growth in *Solanum tuberosum*, might prove profitable. At the same time, it was deemed of interest to follow the changes in starch and sugar content at the different stages, and thus determine whether any correlation exists between diastase activity, growth, and carbohydrate content.

REVIEW OF LITERATURE

The fundamental importance of enzymes in the processes of metabolism has resulted in a voluminous literature on the subject, but a survey of this literature reveals only a very few papers which have a direct bearing on the phase of the subject considered in the present instance. These papers are briefly reviewed below. A larger number have a more or less indirect bearing upon the topic here discussed, and some of these will also be considered.

Probably the first observations on the presence of diastase in the potato were made by Payen and Persoz ('33). They found

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it to be present in the tubers during growth, and also detected its presence in oats, wheat, maize, and rice during germination. They were the first investigators to prepare the enzyme from the extracts of germinated grain, extracting the latter with water and precipitating the ferment by means of alcohol. To this alcoholic precipitate they applied the term diastase, which has persisted since that time. Subsequent investigation showed diastase to be present not only in tissues in which starch normally occurs as a storage product, but also in some where the reserve materials are stored as sugar. Thus, in 1878, Baranetzky found diastase present in the roots of carrots and turnips, which contain no starch. He also found it in the leaves and stems of several plants and in potato tubers. From this evidence, he suggested that diastase was probably universally present in living cells, and later work has practically confirmed this opinion.

Müller-Thurgau ('82) showed that under certain conditions the amount of cupric-reducing substances in leaves increased at the expense of the starch present. He also found ('85) that exposure of potato tubers to a temperature of 0° C. for a month resulted in an accumulation of sugars, with a corresponding loss of starch. Contrary to popular opinion, it was determined by him that no sugar was formed in potatoes which were actually frozen. He has also found that when potatoes which had become sweet by exposure to low temperatures were placed at a temperature of 8–10° C. the sugar disappeared. Müller-Thurgau considered these phenomena to be due to an enzymic process, which, while more rapid at high temperatures, occurs also at low temperatures. According to his ideas, the lessened respiration at low temperatures, entailing the use of less sugar, together with an inhibition of re-formation of starch from sugar—which re-formation takes place rapidly at high temperatures—allows the sugars to accumulate when potatoes are kept at 0° C.

Brasse ('84) is considered by Brown and Morris ('93) to have been the first to prove conclusively the presence of diastase in leaves. He examined the leaves of the potato, dahlia, beet, tobacco, and some other plants, and measured the rate of the activity of the diastase obtained from extracts by precipitation with alcohol. This product was allowed to act upon starch

paste, the rate of activity being determined by the cupric-reducing power of the solution. He used chloroform to inhibit the growth of microorganisms during the time the enzyme was allowed to act, being one of the first to pay attention to this point.

The relative amounts of different carbohydrates in plants often bear some relation to enzyme activity; therefore, the early work of Hungerbühler ('86), who estimated the carbohydrates at different times in growing potatoes, is of interest here. The following figures give the amounts of reducing sugar, invert sugar, and starch, expressed in percentages of the dry weight, found by him in the tubers at different times:

	June 23	June 30	July 7
Reducing sugars	6.4	.32	.72
Invert sugar		4.50	4.69
Starch	56.7	61.30	66.30

Schulze and Seliwanoff ('88) showed the amount of sucrose in immature potatoes to be especially high. Brown and Morris ('90) determined the amounts of diastase present in barley grains at three stages in their development. The results were as follows:

	Relative diastatic activity
Endosperm half developed	4.4
Endosperm two-thirds developed	7.8
Endosperm completely developed	9.7

These results are in line with the idea that the appearance and increase of the enzyme are related to the formation and nutrition of the embryo.

The same authors studied the germination of barley grains and found the amount of diastase to increase markedly during the process. The diastatic activity of the barley embryos after different periods of germination was found to be as follows:

	Relative diastatic activity
Embryos, dissected from grains which had soaked in water for 24 hours	Trace only
Embryos from same barley, but germinated for 3 days on 5 per cent gelatin. Diastatic activity of embryos plus substrate	.1186
Same, except germinated for 4 days	.1634
Same, except germinated for 6 days	.2432

There has, in general, been agreement among investigators as to the occurrence of diastase in plant tissues, but a notable exception in this respect was Wortmann ('90), who concluded from his experiments that the dissolution of starch in plants is brought about directly by the protoplasm and independently of the diastase present. This conclusion was based on experiments made by him which showed diastase to be absent from most leaves, and to occur in others in such small quantity and in such a state as to exhibit only very feeble activity. Even in those cases where this slight activity was manifested it would not, according to him, account for the transformation of starch which actually takes place.

It is of interest in this connection to note that Schimper ('85) had found that starch-free leaves of *Allium* were much less diastatically active than the leaves of *Tropaeolum*, which contain much starch. The comparatively recent work of Bradley and Kellersberger ('12) in this particular should also be mentioned. They found that the leaves of many different species of plants varied greatly in their diastatic content, some, such as the bayberry, onion, and leek, giving so little reaction as to make the presence of the enzyme doubtful.

Brown and Morris ('93) could not agree with the theory of Wortmann, referred to above, that the protoplasm and not an enzyme is directly responsible for the transformation of starch in leaves, but believed, as did Baranetzky, that diastase is universally present in these organs, and that dissolution of starch is brought about by it. They criticized Wortmann's work, chiefly on the ground that he used the clear filtered leaf extracts in his experiments and did not take into consideration the tannins of the leaves, which they found to interfere greatly with the extraction and activity of the enzyme. The clear filtered leaf extract was found by them to possess much less activity than the same extract before filtering.

This work of Brown and Morris is perhaps the most comprehensive and conclusive that has been carried out upon the presence of diastase in plants. They determined the activity of the enzyme in all cases by the addition of .5 gm. of finely powdered, air-dried leaves to 50 cc. of a 2 per cent starch solution

containing 5 cc. of chloroform per liter as preservative. The digestion was carried on for 48 hours, after which the cupric-reducing power of the solution was determined. Their experiments showed diastase to be present in all leaves examined and that it always possessed a decided activity, sufficient even in the cases of the lowest diastase content to transform more starch than the leaves ever contain at one time. These investigators may therefore be considered to have added further evidence in support of the view of Baranetzky, referred to above, that all living cells contain diastase. Only a few facts remain which render it somewhat doubtful. Duggar and Davis ('14), in this laboratory, were unable to demonstrate the presence of the enzyme in *Fucus*, and Bradley and Kellersberger ('12) were doubtful of its presence in such plants as onions, leeks, and certain mushrooms. Failure to find diastase or invertase in the flesh of ripe apples is also reported by Thatcher ('15).

The comparative tests of diastatic activity made by Brown and Morris, referred to above, showed it to be especially high in leguminous plants, while the members of the *Liliaceae* proved to be poor in the enzyme, corresponding with the small amount of starch they contain. To give an idea as to the relative diastatic activity of the leaves of different species, as determined by them, the following plants are selected from the large number reported:

Plant	Relative diastatic activity
<i>Pisum sativum</i>	240.30
<i>Trifolium pratense</i>	89.66
<i>Solanum tuberosum</i>	8.16
<i>Lycopersicum esculentum</i>	6.57
<i>Allium Cepa</i>	3.76
<i>Hydrocharis Morsus-ranae</i>	.27

Attention is directed to the position of the potato in this table, which indicates a comparatively low diastase content.

When the diastatic activity of a set of half-leaves gathered at one time during the day was compared with that of the corresponding half-leaves picked at another time during the same day, it was found to vary considerably. They consider the figures obtained in the last-mentioned case to indicate that the condi-

tions favorable for starch formation are not favorable for enzyme activity and vice versa, since the enzyme activity was much higher at night than during the day. These findings led Green ('97) to investigate the effect of light upon diastase. He found the activity of the enzyme to be considerably decreased by exposure to sunlight, the deleterious effect varying with the intensity of the illumination.

Brown and Morris also demonstrated that diastase, from certain leaves at least, will hydrolyze solid starch, a point disputed by Wortmann. They concluded, too, that their experiments established almost beyond doubt, that, while protoplasm may exert some influence upon the action of diastase upon starch in the early stages of the action, the dissolution of starch is mainly brought about by the enzyme diastase.

In addition to their investigations of diastase in leaves, these authors also worked upon the carbohydrate content of leaves. They concluded that cane sugar, dextrose, levulose, and maltose are present in leaves, but failed to find any pentoses. The amounts of these sugars were found by them to increase in the light and to decrease in the dark, with the exception of levulose, which in many cases followed a reverse procedure. The increase in levulose in the dark was attributed by them to the inversion of cane sugar.

Finally, these authors believed "starvation" of the cell to induce the dissolution of starch through the formation of diastase, and concluded that of the carbohydrates disappearing from leaves in the dark, maltose and levulose contribute most to the respiratory requirements of the cells. This idea of diastase secretion being induced by starvation of the cell is concurred in by Effront ('02, p. 139), who says: "The secretion [of amylase by barley embryos] is always abundant when the germ is found in poor nutritive condition, and it is checked as soon as an assimilable substance appears."

The work of Meyer ('85) was briefly reviewed by Brown and Morris in connection with their work on the carbohydrates of the leaf. Meyer attempted to ascertain if the leaves of such plants as *Allium Cepa*, which store no starch, contain any other cupric-reducing carbohydrates comparable in amount with the

starch of other leaves. He found that plants which store starch abundantly contain comparatively little soluble reducing and non-reducing carbohydrates in their leaves, while others, such as *Allium Cepa*, *Iris germanica*, and *Gentiana lutea*, which store no starch, accumulate relatively large quantities of soluble reducing substances in their leaves. These reducing substances, according to him, appear to be regulated by the same laws as starch in relation to their appearance and disappearance in light and darkness.

The formation and distribution of diastase in the potato tuber was investigated by Prunet ('92), who observed during germination that diastase activity was greater toward the "seed end" of the potato than elsewhere. This corresponds with the greater development of shoots from that region. He found also a correspondence between the sugars present in the different portions of the tuber and the amount of diastase.

Green ('99) states that in some experiments carried on by him in 1893 he found diastase to be present in the pollen of many plants. The amount usually increased considerably at the onset of germination, and appears, it is stated, "to accompany the tube as the latter elongates, which suggests a formation not only in the grain but in its tube also." As the pollen loses with age the power of germination, it also loses its diastase, which is an indication of the part played by the enzyme in germination. This, Green suggests, is comparable with the influence exerted by diastase in the germination of the potato.

The effect of enzymes upon seed germination was the subject of some study by Waugh ('98). Employing solutions of diastase and some other enzymes, he found that the percentage of germination of old tomato seed was considerably increased, in the case of diastase, where the seeds were soaked in the solution for several hours. Taka-diastase gave somewhat higher, but not as uniformly favorable, results as malt diastase. Trypsin also gave good results in some cases.

The work of Keitt ('11) on the formation of sugars and starch in the sweet-potato led him to believe that in the very immature tubers the sugar might be present either as glucose or sucrose, dependent perhaps upon meteorological conditions. He found

the total sugars to decrease gradually as the plants matured, while the starch apparently increased until the vines were killed by frost, at which time it was at its maximum. Killing of the vines by frost was followed by a breaking down of starch with a tendency toward the formation of sucrose. The following is a typical example of his analyses, in which figures are given for the small immature tubers harvested on August 28, and for successive stages until the potatoes were mature:

Date	Starch	Glucose	Sucrose
Aug. 28.....	17.88.....	.61.....	4.18
Sept. 7.....	14.91.....	1.58.....	1.82
Sept. 18.....	17.61.....	1.88.....	1.91
Sept. 29.....	19.92.....	2.30.....	1.43
Nov. 18.....	17.82.....	1.53.....	2.35

(A freeze occurred on Nov. 6 which killed the vines.)

It has been generally assumed that transformations brought about through the agency of enzymes are reversible; that is, diastase which hydrolyzes starch to maltose also acts as the synthesizing agent in the production of starch. The hydrolytic processes have, of course, received most attention, but have not been exclusively investigated. The synthetic aspect of the problem was attacked by Bradley and Kellersberger ('12). In view of the results obtained, they were unwilling to make any general deductions as to the relation between the diastatic activity of a tissue and its starch-storing function, though their determinations seemed to give considerable support to the view that the enzyme is the synthesizing agent. One reason for their hesitancy in formulating a conclusion was their inability to explain the fact that fresh mushroom tissues, rich in a glycogen-like carbohydrate, when allowed to act upon a soluble starch solution for five days, showed no evidence of diastase activity, while the same tissues ground up with alcohol, washed with ether, and dried, developed considerable activity. They thought this might be explained by the fact that the treatment activated a proenzyme, but if such were the case, they could not understand why in a rapidly growing mushroom in which the glycogen transfer must necessarily be rapid diastase should not be present, if that enzyme is necessary for synthesis.

Some other facts also introduced an element of doubt into the matter. Beets and mangels, which store their carbohydrates as sugar instead of starch, were found to possess highly diastatic leaves, while the roots contained none of the enzyme. No trace of starch could be found in roots of the radish, yet it was one of the most diastatically active tissues studied. Likewise the potato tuber, which is, of course, particularly rich in starch, proved to be poor in diastase. The leaves of different species of plants were found by them to vary greatly in diastase content. The enzyme appeared most abundant in leaves where, to quote them, "starch is never stored permanently, but where it may be found in small amounts during photosynthesis." These statements do not agree very well with that made by Haas and Hill ('17, p. 369), which is as follows: "The amount of diastase is always greater in starch leaves than in sugar leaves, and the same holds for insolated leaves containing much starch, as compared with shaded leaves containing little or no starch."

In studying this problem of carbohydrate synthesis in plants, Bradley and Kellersberger made some determinations of diastatic activity which are of interest in connection with the present problem, and a few of these are given below. The figures have been recalculated on the basis of 100 which represents the greatest activity reported.

Plant	Relative diastatic activity	Amount of starch content
Pea, seeds, medium	100.0	Medium
Pea, seeds, mature	50.0	Abundant
Corn, seeds, young	10.0	Medium
Corn, seeds, medium	6.7	Abundant
Corn, seeds, mature	7.7	Abundant
Corn, cob, young	7.7	Small
Corn, cob, mature	7.7	Small
Corn, husk, young	25.0	None
Corn, husk, mature	8.3	None
Potato, root, medium	2.8	Some dextrin
Potato, tuber, medium	2.9	Very abundant
Potato, leaf, medium	75.0	None*

* Potato leaves generally contain starch, but in this case they were obtained in the early morning before photosynthesis had taken place to any extent.

The diastatic activity of both potato and onion leaves was determined by Brown and Morris ('93) and by Bradley and Kellersberger. Thus it is possible to compare, by means of the values given for onion leaves, the activities of the potato leaves as found in the two cases. Different units, of course, are used to express the enzyme activity in the two cases.

Determination made by	Relative diastatic activity	
	Potato leaves	Onion leaves
Brown and Morris.....	8.2.....	3.80
Bradley and Kellersberger.....	75.0.....	1.05

It is seen from the above that the relative activity in the case of the potato, as found by Bradley and Kellersberger, was much higher than that found by Brown and Morris. This is doubtless partly due to the fact that the former authors made their determinations by means of the cupric-reducing power of the solutions, while the latter employed the iodine reaction. It illustrates the wide difference that often occurs in the results obtained by different workers along this line. In this case, however, the difference is not one of direction, but of degree only.

The physiological changes taking place in the potato during its rest period have been studied to a considerable degree by Appleman ('11, '16). He found, as did Müller-Thurgau and others, that glucose and sucrose accumulate in potatoes which are stored at 0° C. Diastase activity was also greater at the end of 2 and 4 weeks in the cold-storage potatoes than in those stored at room temperature, but at the end of 6 weeks practically no difference could be detected, as, he explains, this was near the end of the rest period of the variety used in this work. Appleman ('11) says that "the increased diastatic activity is probably due to greater activation of zymogen by free acids which are liberated by the greater permeability of protoplasmic membranes at low temperatures."

It is well known that potatoes will not germinate for several weeks after being harvested, apparently because certain changes known as "after-ripening" must first take place. In attempting to determine the nature of these changes, Appleman ('16) reached the conclusion that carbohydrate transformations in the potato tuber during its rest period must not be considered

after-ripening processes, but simply due to, and dependent upon, changing temperature. He did not find an increase at the time of sprouting in the reducing or total sugars in the case of potatoes which, since harvesting, had been stored under growing conditions; nor was there any difference in the sugar content of the seed and stem ends of the tubers at the beginning of sprouting. The diastase activity, he states, was uniformly greater in the extract from the seed end, but there was no appreciable increase in diastatic activity in either end during the rest period, in spite of the fact that sprouting begins much earlier in the seed end. From this evidence, the conclusion is reached that the "cessation of the rest period is not due to a gradual increase in diastatic activity."

Appleman ('16) found further that sprouting could be brought about at any time during the rest period by removing the skins of the tubers. There was still earlier sprouting in tubers which were cut in half transversely, those buds near the exposed surface starting first. Subdued light and a treatment consisting of wrapping the potatoes with cotton saturated with hydrogen peroxide were both effective in shortening the rest period in new tubers with skins not highly suberized. The author believed the shortening of the rest period in all these cases to be correlated, not with water absorption, but with increased absorption of oxygen.

In his studies of the rest period of plants, Howard ('15) found that when certain agents, such as etherization, desiccation, warm water bath, etc., were used to break the rest period, the diastatic activity of the treated tissues was increased, such increase agreeing in each case with the extent to which the treatment broke the rest period. Corresponding with this increased diastase activity, the amounts of soluble reducing sugars were also found to increase within 24 hours after application of the treatment, provided the treatment was applied during the early winter. Treatments given later in the season were found to have very little effect.

The work of Howard is confirmed to some extent by that of Bonns ('18) in this laboratory. The latter, in studying the effect of etherization upon enzyme activity in corms of *Gladiolus*,

found increased hydrolysis of starch by the enzyme, following treatment with the anaesthetic.

Butler ('13) observed, by means of the coloration produced in slices of tubers by boiling them in Fehling's solution, and also by analyses, the sugar content in potatoes during the rest period and at the time of sprouting. He makes the statement that the accumulation of sugar in the tubers has no particular physiological significance as regards germination. He calls attention to the fact that tubers which germinate at a relatively low temperature contain more sugar than those which germinate at relatively high temperatures, and also that those which are allowed to sprout in the soil have a higher sugar content than those sprouted in the cellar. Certain sprouting potatoes examined by him were found to contain no sugar, though the sprouts themselves contained a considerable amount. The same potatoes, after being stored in an ice chest for 20 days, were found to have accumulated a considerable amount of sugar, while the sugar in the sprouts apparently remained constant. At the same time potatoes taken from storage where the temperature had not fallen below 6° C. showed considerable sugar in both tuber and sprouts. His illustrations show that stored potatoes kept under similar conditions were quite variable as to sugar content, some not containing sugar at all or in the cortex only, others in the medulla only, and still others in both cortex and medulla.

According to some further results obtained by Butler, there is less sugar in the vicinity of sprouting eyes than elsewhere, and by analyses he found the sugar content of the seed ends of sprouting potatoes to be small, as a rule, and to increase toward the stem end. This is at variance with the results of Prunet ('92), who found the greater amount of sugar in the seed end of the tuber, and also with those of Appleman ('16), who found no appreciable difference in the sugar content of the opposite ends of those potatoes examined by him.

Butler believes that the greater metabolic activity at the seed end of the tuber, both during the rest period and at germination, is unfavorable for an accumulation of sugar at that point, and concludes from his data that there is "little if any translocation from remote to budding parts, even in germinating potatoes."

The author states, finally, that since the distribution of sugar in resting and germinating potatoes is not essentially different, its appearance in quantity at the time of germination should be ascribed, partly at least, to metabolic changes induced by other agencies.

Doby and Bodnar ('15) investigated diastatic activity in tubers from healthy potato plants as compared with that in tubers from plants affected with leaf roll. They found the absolute value of the diastase (diastase plus zymogen) to be the same in healthy and diseased potatoes, but, in general, more zymogen was found present in healthy than in diseased tubers. These workers also investigated the presence of zymogen in the extracted juice of potatoes. They observed that the diastatic activity of the juice increased upon standing, which fact they attribute to the rapid conversion, in the extracted juice, of zymogen into amylase. Fresh juice, which was quite active to begin with, did not increase to the same extent as did other juice in which diastase was less active in the beginning. Less zymogen was found to be present in tubers in the middle of the rest period than at other times. It began to increase during the first half of January, and a corresponding increase in diastatic activity was observed at the same time. In the spring only small amounts of zymogen were found, since at that time most of it had been changed into active diastase.

It was believed by these investigators that the diastatic activity of fresh juice depends chiefly upon the stage of development of the tuber, but that it may also be affected by the kind of potato used and by climatic and cultural conditions during growth. The stage of the rest period must also be taken into consideration when determinations are made during that time. Enzyme activity was found to be almost entirely independent of the size of the tubers. This calls attention to the necessity for the consideration of these factors in relative diastatic activity determinations, and emphasizes the importance of a uniform procedure as regards the time that elapses between the different operations necessary in such determinations, as well as the importance of using material, the different stages of which have developed under known and comparable conditions.

One of the last-mentioned authors, Doby ('14), carried out some experiments to determine the effect of the different sugars upon the amylase of potatoes. From the results obtained, he concluded that the action of the enzyme is inhibited by the sugars in the following order, maltose being most effective,—maltose, glucose, fructose, arabinose, galactose, mannose.

He further concluded from his experiments that it is quite probable that the action of amylase in a natural enzyme system is restricted, in the first place by the decomposition products of the substrate, and in the second place by other sugars which may be present.

Since maltose is the final product in the hydrolysis of starch by amylase, it might seem that the activity of the enzyme would soon be checked by the accumulation of this sugar, but Davis, Daish, and Sawyer ('16), in their extensive work upon the formation and translocation of carbohydrates in plants, found that maltose was immediately transformed into hexose by the maltase present, so that there is only a trace of this sugar, in leaves at least, at any one time. Moreover, Norris ('14) states, as a result of experiments upon the factors influencing hydrolysis of glycogen by diastase, that while the mixed products of hydrolysis have a marked retarding influence on the velocity of the reaction, maltose alone has very little effect.

In studying the effect of activators upon the diastase of potatoes, Doby and Bodnar ('15) found, among other things, that the boiled juice exerted a considerable activating influence. Effront ('02) made a similar observation in regard to malt diastase.

Carbohydrate transformations in the sweet-potato resemble to a degree those in *Solanum tuberosum*. Hasselbring and Hawkins ('15, '15a), and Hasselbring ('18), from their studies on this subject, reached the conclusion that, while sweet-potatoes contain only very small amounts of sugar during growth, the transformation of starch into sugar begins immediately after harvesting or upon the killing of the vines by frost. The same changes were found to occur whether the tubers were stored in cellars or remained in the ground. These changes involved, according to the authors, the transformation of starch to reducing sugars

and the subsequent synthesis of cane sugar from these. The formation of sugars was observed to be rapid at a high temperature (30° C.), more gradual at temperatures of 11.7 – 16.7° C., while lowering the temperature to 4° C. again accelerated the process.

Hasselbring and Hawkins ('15) also found, in the course of further experiments, that the carbohydrate content of the two halves of a potato, which had been split longitudinally, was not the same in all cases. While the analyses of the two halves of a freshly dug tuber agreed closely, differences of from 1 to 16 per cent were found in corresponding halves of tubers which had been kept for a time. These differences were found regardless of the temperature at which the potatoes had been kept. These authors consider their work to confirm and extend the investigations of Keitt, referred to previously.

The extensive work of Davis, Daish, and Sawyer ('16) on the carbohydrates of the mangold leaf, and that of Davis and Sawyer ('16) on the carbohydrates of potato leaves are perhaps the most recent contributions along this line. These investigators not only secured much valuable information regarding distribution of sugars and starch at different hours of the day in the leaves mentioned, but also developed methods for the quantitative determination of these substances in plant material. These methods, while they may, according to Jørgensen and Stiles ('17), be subject to certain errors, are doubtless the most reliable to be found at the present time.

Some of the results obtained in this work were as follows: (1) Starch was found to be entirely absent from mangold leaves except in the early stages of growth, and maltose was never present, either in the leaves, stalks, or midribs, at any time of the day or night. (2) Sucrose was present in the leaf in excess of hexoses in the early stages of growth, but later, when sugar was being stored in the root, the hexoses predominated. The latter sugars were also more abundant than sucrose in the midribs and leaf-stalks. Sucrose was therefore concluded to be the first sugar formed in photosynthesis, being transformed into hexoses for the purpose of translocation. Pentoses were found to form a small proportion of the sugars in the tissues. (3) The

hydrolysis of starch was believed to be effected by a mixture of enzymes, containing maltase in relative excess, so that the transformation of starch to dextrose was complete.

The conclusions in regard to sucrose are in agreement with the views of Brown and Morris ('93). The absence of maltose, however, is contrary to the findings of the latter authors, and is due, according to Davis, Daish, and Sawyer, to the fact that enzyme activity was not checked promptly in the material examined by them. Disagreement also exists in regard to pentoses, Brown and Morris not finding these sugars present.

MATERIALS AND METHODS

Potatoes of the Irish cobbler variety were used in this work. They were grown on a bench in the greenhouse, and, as is usually the case under such conditions, the mature tubers were small, the largest being only 6 cm. in diameter. It was the aim at the outset to make analyses of the tubers for enzyme activity, reducing sugars, sucrose, and starch, beginning when the young potatoes had attained a diameter of 1 cm., and making later determinations corresponding with each increase of 1 cm. in diameter until maturity. This plan has been followed with the exception that it was not possible to obtain sufficient tubers of the first size (1 cm. in diameter) for the determinations of both diastase activity and carbohydrate content, but enough were found for the enzyme activity determinations. Some analyses were also made of seed potatoes.

Upon reaching the proper size the potatoes were harvested, washed free from all particles of dirt, and dried with a towel, after which the analysis of the material was begun without delay. These and all other operations were carried out as quickly as possible, so as to avoid errors which might be due to delay in handling.

This procedure may be objected to by some on the ground that size of the potato tuber is not necessarily correlated with stage of development. In these experiments, however, the first four sizes (1-4 cm. in diameter, inclusive) were all dug at the same time, while the vines were still actively growing, and the 5- and 6-cm. sizes were secured about two weeks later. All

these tubers, except the largest size, which was practically mature, were still in an active, growing condition, and it is believed that they really represented not only different sizes but also different stages of development. This should be especially true in the case of the enzyme activity determinations, as it was necessary in making these to use a rather large number of the tubers to obtain the juice needed, thus providing a composite sample.

Diastase activity.—Two or three methods of obtaining the enzyme were tried before a satisfactory one was found. When the potatoes were sliced and dried by means of alcohol and acetone, according to the method used by Davis ('15), and then finely ground in a mortar, extracted with water, and the enzyme precipitated with 95 per cent alcohol, the diastase so obtained was apparently not active enough to make the method satisfactory.

A method whereby the enzyme was precipitated by alcohol from the pure juice of the potato was then tried. The juice was obtained by quickly grating the tubers, grinding the material in a mortar with carborundum, squeezing the juice out by means of a tourniquet of cheese-cloth, and finally filtering through asbestos and filter-paper. The filtering was done in order to remove the starch present. The enzyme obtained from this juice by precipitation with alcohol apparently possessed no activity whatever, and it was finally decided to use the juice itself, as preliminary tests had shown it to be quite active diastatically. The fact that the juice of the potato has considerable activating influence upon the diastase, as found by Doby ('14), was confirmed, and this no doubt accounts for the inactivity of the alcohol-precipitated enzyme.

In determining diastatic activity, 20 cc. of the fresh juice were added to 100 cc. of a .25 per cent soluble starch solution in a 250-cc. Erlenmeyer flask, 2 cc. of toluol added, and the flasks placed in the incubator and kept at 45° C. for 12 hours. At the end of this time the flasks were placed in boiling water for 10 minutes to kill the enzyme.

At the same time checks were made on the above by substituting 100 cc. of water for the starch in two flasks, incubating one

of these for the same length of time and placing the other in boiling water for 10 minutes to kill the enzyme. The diastase activity determinations therefore included the following in each case: one flask containing juice plus starch, incubated 12 hours; one flask containing juice plus water, incubated 12 hours; one flask containing juice plus water, boiled at once to kill the enzyme. The cupric-reducing power of the solution in the first flask less that of the solution in the third flask is considered to represent the activity of the enzyme.

Some preliminary work seemed to indicate the presence of an activating agent in the potato juice, in the absence of which the enzyme possessed little or no activity. To determine this point, some experiments were carried out according to the following procedure: The enzyme, which was precipitated from about 200 cc. of potato juice by the addition of three volumes of 95 per cent alcohol, was collected on filter-paper and dried, and then dissolved in 100 cc. of water and filtered, after which it was ready for use. Another 200-cc. sample of the juice was boiled, killing the enzyme and precipitating the proteins. This was also filtered before using.

In making the determinations, flasks were prepared as below. The same amounts of diastase solution and boiled juice were used in each instance, water being added to make the volume the same in all cases. Two per cent of toluol was used as a preservative.

One flask containing starch plus enzyme.

One flask containing starch plus enzyme plus boiled juice.

These were incubated for varying periods of 12 to 24 hours at 45° C.

One flask containing enzyme plus boiled juice.

This was boiled at the beginning of the experiment to kill the enzyme.

After incubation the cupric-reducing powers of the solutions were determined and these accepted as an index of the diastatic activity.

Extraction of sugars.—For the sugar determinations, 50 gms. of the green tubers (from the same lot used in the enzyme activity determinations) were weighed out and immediately sliced

into thin slices which were dropped into about 200 cc. of boiling 95 per cent alcohol containing 1 per cent of strong ammonia. After boiling for 10 or 15 minutes, this material was transferred, as nearly quantitatively as possible, to a Soxhlet extraction apparatus and extracted for 18 to 24 hours. After washing out the extraction apparatus with alcohol, this extract, which usually amounted to 400–500 cc., was then evaporated *in vacuo* at a temperature of 35–40° C., leaving finally a volume of 40–50 cc. This extract always contained a certain amount of loose starch, which was removed by centrifuging, after which the extract was made up to 200 cc. with doubly distilled water. When the analyses could not be made at once, 2 per cent toluol was added as a preservative.

This method of extraction and evaporation *in vacuo* is essentially that described by Davis, Daish, and Sawyer ('16) and is quite effective in removing the sugars. The necessity for transferring the material from one vessel to another several times during the process introduces the possibility of error, but with careful handling this is small.

Estimation of reducing sugars.—In making the determinations of reducing sugars, the method described by Shaffer ('14) was employed, with slight modifications. Forty cc. of the sugar solution were used instead of the 10 cc. recommended. This was done on account of the dilution, which made duplicates difficult to obtain when only 10 cc. were used. Five cc. of water and 5 cc. of colloidal iron brought the total to 50 cc., instead of 40 cc. as in the original method used by Shaffer. After removing the iron by centrifuging and filtering, two 15-cc. samples of the filtrate were used for the determinations, each sample corresponding to 12 cc. of the original sugar solution. The dissolved cuprous oxide was titrated with $\frac{N}{20}$ potassium permanganate, and duplicates checked within .1 or .2 cc. of permanganate.

Determination of sucrose.—For the determination of sucrose, the method used was the same up to the point where the two 15-cc. samples were measured out. Then 1.5 gms. (10 per cent) solid citric acid were added to each sample, as recommended by Davis and Daish ('13) for the inversion of sucrose. The samples

plus the citric acid were introduced directly into 50-cc. centrifuge tubes and placed in a boiling water bath for 10 minutes, after which the acid was neutralized to phenolphthalein with normal sodium hydroxide, Fehling's solution added, and the balance of the process completed by the Shaffer method. The sucrose was then determined as glucose by the increase in the cupric-reducing power of the solution, due to inversion of the sucrose.

Starch determinations.—The material from which the sugars had been extracted was used in the starch determinations. This was usually sufficiently dried by standing in the air for a few hours so that it could be ground up finely in a mortar. It was then carefully weighed and exactly $\frac{1}{5}$ of it measured out, made into a paste by adding a small amount of water, and the paste poured into 400 or 500 cc. of boiling distilled water. Then the starch which had been centrifuged out of the sugar solution was suspended in 100 cc. of distilled water, and 20 cc. ($\frac{1}{5}$) pipetted out and added to the other. The whole was then gelatinized by boiling under a reflux condenser for two hours, cooled, and made up to one liter with distilled water, 2 per cent toluol being added to prevent the growth of microorganisms. One-tenth gm. Taka-diastase was then added to 100 cc. of the solution, which was well shaken during sampling, and this was incubated at 45° C. for 24 hours. Under these conditions, according to Davis and Daish ('14), hydrolysis of the starch is complete, it having been broken down into glucose and maltose, which exist in a definite ratio in the solution. These sugars were then determined as glucose. The value found, of course, is not the true starch value, but may be used as a basis for comparison, which is all that is demanded in this work.

RESULTS AND DISCUSSION

Diastase activity.—The results of the diastase activity determinations are given below (table 1). These results are, of course, comparative only. The figures in the first three columns represent the number of cubic centimeters of potassium permanganate used in titrating the dissolved cuprous oxide in one sample, while the relative enzyme activity (the difference between the figures in the first and third columns) is given in the

fourth column. The latter is expressed in terms of 100, which is assumed to be the value of the greatest activity observed.

TABLE I
DIASTASE ACTIVITY IN RELATION TO STAGE OF DEVELOPMENT OF
POTATO TUBERS*

Stage of development (diameter in cms.)	No. cc. $\frac{N}{20}$ KMnO ₄ used in titrating Cu ₂ O			Relative diastatic activity
	Juice+starch (incubated)	Juice+water (incubated)	Juice+water (boiled)	
1.	10.7	Lost	8.0	66
2	13.0	11.4	10.1	71
3	12.8	10.0	8.7	100
4	9.9	7.1	6.5	83
5	6.2	3.5	2.2	98
6	5.7	2.9	1.7	98
Seed potato	11.0	8.4	7.8	78
Seed potato	8.2	6.6	6.5	41
Seed potato	9.6	7.3	7.1	61

* In the growing potatoes, not less than 500 gms. of the tubers were used, in each case, as the source of the juice.

It appears from these figures that advance in stage of development is accompanied by an increase in diastatic activity. The author is inclined to believe that the high value obtained for tubers 3 cm. in diameter is perhaps abnormal and without particular significance.

The diastatic activity of three samples of seed potatoes is also given for purposes of comparison. The first two were taken from the lot that was used for seed, while the third represents a different variety. They had been kept in ordinary storage up to the time the analyses were made. It will be observed that their diastatic activity is considerably lower than that of most of the growing tubers. It is rather surprising that the two samples from the same lot of stored tubers should possess such widely different diastatic activities as 78 and 41.

The second column in table I is included to show the increase in reducing sugars which occurs when the juice alone (diluted

with water) is incubated. If the figures in the third column, which represent the diluted juice boiled at the beginning of the experiment to kill the enzyme, are compared with those in the second, it will be seen that there is a considerable increase in reducing sugars. This is probably due to conversion, by the enzyme, of dextrins in the juice, to reducing sugars. The differences in this respect are less in the case of the seed potatoes, which corresponds with their lower enzyme activity.

The activating effect of boiled potato juice upon the diastase of potatoes has been observed by Doby ('14). The table given below (table II) shows some results obtained by the writer on this point. The figures represent cubic centimeters of potassium permanganate used in titrating the dissolved cuprous oxide. The "enzyme" used is that obtained by precipitation with alcohol, as has already been described.

TABLE II
EFFECT OF BOILED JUICE UPON THE ACTIVITY OF POTATO DIASTASE

Sample	No. cc. $\frac{N}{20}$ KMnO_4 used in titrating Cu_2O			Difference due to enzyme in presence of boiled juice
	Enzyme+ starch	Enzyme+ starch+boiled filtered juice	Boiled filtered juice	
1	0	4.1	2.8	1.3
2	0	5.6	4.3	1.3
3	0	2.2	.6	1.6
4	.4	26.0	24.6	1.4
5	.3	5.9	4.9	1.0

From the figures given, it seems that the activity of diastase of potatoes depends upon some substance contained in the juice, but not precipitated by alcohol. It is well known that a number of substances have an activating effect upon diastase, but it has been generally considered that the enzyme when alone would act upon starch, and as far as the author is aware, no one has before presented evidence to show that this is not always the case.

In the first tests made, no activity whatever was observed when the enzyme alone was added to the starch solution, but in

the last two a very small amount of starch was hydrolyzed after 24 hours. While the latter results must be considered positive, the figures are so small as to be almost within the range of experimental error.

Since the activity of diastase is accelerated by small amounts of acids, it was thought that the activating effect of the boiled juice might possibly be due to its hydrogen ion concentration. Accordingly, determinations were made in two cases which gave an H ion concentration of P_H 5.7 and P_H 6.2. This very slight acidity could hardly influence the activity of the enzyme. However, a solution consisting of mono- and dipotassium phosphate having the same P_H value was substituted for the boiled juice in each case, but failed to have any activating effect.

Another point of some interest in regard to diastase activity was incidentally observed in the course of these experiments, that is, the direct effect of the time factor upon diastatic activity of potato juice. Doby and Bodnar ('15) report that when the juice was preserved with toluol and allowed to stand in the dark at 8–10° C. for 24 hours or longer, its diastatic activity increased. As stated, the results reported here were obtained incidentally, without any effort to provide Doby and Bodnar's conditions, and are given for what they are worth (table III). The juice used was preserved with 2 per cent toluol and stood at room temperature in partial light.

TABLE III
EFFECT OF THE TIME FACTOR UPON THE DIASTATIC ACTIVITY OF
POTATO JUICE

Sample	Relative diastatic activity	
	Fresh juice	After standing 24 hrs.
1	98	66
2	120	59

These results indicate that under the conditions which obtained in the experiment there is a marked decrease in the diastatic activity of the potato juice on standing 24 hours. This is directly opposed to the conclusions of Doby and Bodnar, who,

however, it must be remembered, kept the juice under different conditions. In view of the results given here, it would seem desirable to repeat the work of the latter authors, employing the conditions under which their results were obtained.

Carbohydrate content of potatoes.—The carbohydrate content of the tubers used in this work is given in table iv. The figures given show the amounts of reducing sugars, sucrose, and starch, all determined as glucose, present in 50 gms. (green weight) of the freshly dug tubers at each stage of growth. No analyses were made of tubers 1 cm. in diameter, due to the fact, as stated before, that while enough of the potatoes of this size were available for the diastase activity determinations, there was not sufficient quantity to make also the sugar and starch determinations.

The results given in table iv show a gradual decrease, with advance in development, in the amount of reducing sugar present, which is accompanied up to a certain point by a corresponding increase in the amount of sucrose. The latter falls off rapidly, however, as the tuber approaches maturity. This is in agreement with the limited data of Hungerbühler ('86), who also found a decrease in the reducing sugars and an increase in invert sugar, with advance in maturity. The results of Schulze and Seliwanoff ('88), who found sucrose to be abundant in the immature tubers, are also confirmed.

TABLE IV.

CARBOHYDRATE CONTENT OF POTATO TUBERS AT DIFFERENT STAGES OF GROWTH

Stage of growth (diameter in cms.)	No. gms. carbohydrate, determined as glucose, in 50 gms. green tubers		
	Reducing sugar	Sucrose	Starch
2	.3854	.4217	6.963
3	.3307	.4395	6.039
4	.2388	.6358	6.236
5	.0774	.6194	7.494
6	.0127	.0924	7.632
Seed potato	.1360	.0722	7.065
Seed potato	.1857	.0675	8.650

The results obtained may also be considered to agree with those found by Keitt ('11) for the sweet-potato. The latter found the total sugars in the sweet-potato tuber to decrease toward maturity, and, from the figures given above, the same tendency appears to exist in the Irish potato. In the case of the seed potatoes, the reducing sugars are present in much larger quantity than in the larger sizes of the growing tubers, while the sucrose is lower than the lowest value obtained in the case of the growing potatoes.

It is somewhat difficult to interpret the sugar relations of the potato tuber, as shown by the figures given, but the following is a possible explanation: Sugars in plants are translocated chiefly in the form of the hexoses, glucose and levulose. When photosynthesis and growth are proceeding at a rapid rate in the early stages of development and when diastase activity is not so great as later on, it would be expected that the translocated sugars (reducing sugars), would be present in the potato tuber in comparatively large amounts. Diastase, acting as a synthesizing agent, would gradually convert the glucose into the storage product, starch, but this alone might not suffice to dispose of all the reducing sugars accumulating in the tuber, so the invertase present may also be conceived to act as a synthesizing agent and transform some of it into a temporary storage product, sucrose, which accumulates up to a certain point. Towards maturity, as the translocation of hexoses becomes less rapid and as the glucose which reaches the tuber is more rapidly converted into starch by the more active diastase, less reducing sugar is found present. As the reducing sugar decreases, some of the sucrose is hydrolyzed to hexose by the invertase, possibly in connection with the general matter of equilibrium relations, and thus a decrease in the sucrose is brought about. Under the influence of the low temperatures which prevail in storage, the starch may be partially hydrolyzed to reducing sugar, some of which may again be built up into sucrose, thus causing an accumulation in the tubers, such as has been observed by a number of investigators.

An examination of the values obtained in the starch determinations shows that the percentage of this carbohydrate increases gradually as the tuber enlarges in size. The relatively high

value found in the case of tubers 2 cm. in diameter does not follow the general trend, and remains unexplained. The two samples of seed potatoes exhibit a rather wide difference in starch content, which, the author believes, is perhaps due to varietal differences or to a difference in the conditions under which the potatoes were kept. The conditions under which the tubers were grown may also have exerted some influence in this respect.

SUMMARY

A review of the literature, bearing directly or indirectly upon the subject of this paper, is presented.

The methods of experimentation are described. They include the determination of diastase activity of potato tubers at various stages of development by adding fresh potato juice to a solution of soluble starch; the extraction and estimation of reducing sugars and sucrose by the comparatively new method of Davis, Daish, and Sawyer; and the estimation of starch by means of Taka-diastase.

The following results were obtained:

Diastase activity and starch content were found to increase with advance in the development of the tubers.

In general, the increase in enzyme activity and starch content of growing potatoes was accompanied by a decrease in the total sugars present.

When the juice of potatoes was preserved with toluol and kept for 24 hours at room temperature, its diastatic activity was found to decrease quite markedly.

Evidence was obtained which indicates that a co-enzyme is necessary in the hydrolysis of starch by potato diastase. The activating agent exists in the juice, and is not destroyed by boiling, nor is it precipitated by alcohol.

It is the opinion of the author that if a number of analyses were made along the lines described and the average of these taken the comprehensive results thus obtained would clear up the points which, in this report, appear somewhat doubtful, and would make it possible to draw definite conclusions.

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